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Gfi1—Cells & Circuits: Unraveling transcriptional networks of development and disease

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Abstract

Purpose of review—The review will integrate current knowledge of transcriptional circuits whose dysregulation leads to autoimmunity, neutropenia and leukemia.

Recent findings—Growth factor independent-1 is a transcriptional repressor with essential roles in controlling hematopoietic stem cell biology, myeloid and lymphoid differentiation and lymphocyte effector functions. Recent work has suggested that Gfi1 competes or collaborates with other transcription factors to modulate transcription programs and lineage decisions.

Summary—Gfi1 is central to several transcriptional circuits whose dysregulation leads to abnormal or malignant hematopoiesis. These functional relationships are conserved from *Drosophila* development. Such conserved pathways represent central oncogenic or “gatekeeper” pathways that are pivotal to understanding the process of cellular transformation, and illustrate key targets for clinical intervention.

Keywords

Growth factor independent-1; HoxA9; Pu.1; transcription; hematopoiesis; leukemia; severe congenital neutropenia

Introduction

Growth factor independent-1 (Gfi1) is a zinc-finger transcriptional repressor that was originally identified in an *in vitro* screen for Moloney murine leukemia virus (MMLV) insertion loci that could progress IL-2 dependent T-cell leukemias to IL-2 independent growth [1]. In fact, the *Gfi1* locus is activated by multiple common insertion loci [1,2], which when considered together reveal *Gfi1* as the most frequently activated gene in

MMLV-induced lymphoid malignancies [3]. In contrast, *Gfi1* is a rare target of MMLV-related retroviruses in myeloid malignancies, perhaps because *Gfi1* suppresses myeloid transformation [4]. Similar to other MMLV targets, *Gfi1* is a critical regulator of hematopoietic development and function (see reviews[5-7]). Moreover, *Gfi1* functions extend to intestinal, neuroendocrine and sensory neural cell differentiation [8-10].

Gfi1 mediates transcriptional repression dependent upon DNA binding and a 20 amino-acid amino-terminal “SNAG” transcriptional repressor domain [11]. *Gfi1* repression function is mediated by the recruitment of a small number of potent chromatin modifying enzymes such as ETO [12], histone deacetylases (HDAC) 1, 2 & 3[12,13], histone lysine methyltransferase G9A [13], lysine specific demethylase-1 (LSD1)/CoREST [14], and Ajuba [15]. While these cofactors function by different mechanisms, they share the ability to interact with *Gfi1* on target specific DNA sequences to gene transcription. Recent work has suggested that *Gfi1* DNA binding sites can overlap or occur in close proximity to those of HoxA9, Pu.1, or C/EBP proteins, and competition or collaboration between *Gfi1* and these proteins induces different transcriptional outcomes [4,16,17]. The focus of this review will highlight *Gfi1* as a central player in transcriptional circuits whose dysregulation leads to neutropenia and cancer.

Gfi1 in Hematopoietic Stem Cell Biology

Gfi1 is required for adult hematopoietic stem cell (HSC) quiescence [18,19]. Deregulation of the *Gfi1* target gene $p21^{cip1/waf1}$ [20] was posited as a partial mechanism to explain the loss of HSC quiescence [18,19]. However, *Gfi1*^{-/-} and $p21$ ^{-/-} display dissimilar lineage negative, Sca1⁺, cKit⁺(LSK) numbers, with *Gfi1*^{-/-} stem/progenitors in active cell cycle leading to decreasing numbers of LSK while $p21$ ^{-/-} induces a decrease in stem/progenitor G₂-M phase [18,19,21-23]. In fact, recent studies have demonstrated that HSC quiescence is a $p21$ -independent function of $p53$ [24]. Notably, while deregulated $p21^{Waf1}$ is not sufficient to phenocopy $p53$ ^{-/-} or *Gfi1*^{-/-} HSC effects, it is formally possible that deregulation might still be a necessary precursor to $p53$ ^{-/-} or *Gfi1*^{-/-} HSC phenotypes.

Recently, $p53$ and the Ets transcription factor Mef/Elf4 have been implicated in controlling HSC maintenance, perhaps through *Gfi1* (Fig 1) [24]. In the absence of Mef/Elf4, a known regulator of HSC [25], LSK display a 10-fold increase in *Gfi1* mRNA [24]. Mef controls the expression of Mdm2, which regulates the stability of the tumor suppressor $p53$. In turn, $p53$ regulates multiple HSC functions including proliferation and apoptosis [26,27]. Interestingly, *Mef*^{-/-} induced *Gfi1* mRNA was entirely dependent upon $p53$, as $p53$ ^{-/-}*Mef*^{-/-} mice had the same 2.5 fold decrease in *Gfi1* as $p53$ ^{-/-} LSK [24]. In agreement with this, $p53$ ^{-/-} HSC show a partial phenocopy of *Gfi1*^{-/-} HSC: decreased LSK cells in G₀, and increased proliferation of LSK. However, while *Gfi1*^{-/-} LSK fail in competitive BM repopulation assays, $p53$ ^{-/-} LSK are competitive [19,24]. This may reflect either hypomorphic levels of *Gfi1* in $p53$ ^{-/-} (as opposed to the complete absence of *Gfi1* in *Gfi1*^{-/-} mice) or a divorce between the previously welded concepts of *Gfi1*^{-/-}-HSC fitness defects and *Gfi1*^{-/-}-increased cell cycle. Whether $p53$ regulates *Gfi1* outside of HSC is not known.

Independent of $p53$, the functional effects of hypomorphic *Gfi1* levels remain to be directly proven. Kuo et al. recently demonstrated that Runx2 induces acute myeloid leukemia in cooperation with Cbfb-SMMHC in mice, and that one effect of Runx2 forced expression was to repress *Gfi1* expression [28]. In addition, Huh et al. demonstrated that *Gfi1* transcript levels were low in patient myelodysplastic syndrome (MDS) samples, but whether this is due to low numbers of progenitors or a functional link to MDS is unknown [29]. We now know that *Gfi1*^{-/-} myeloid progenitors are predisposed to transformation [4], but future

work is needed to rigorously determine the biological sequelae of hypomorphic levels of Gfi1.

Gfi1 in Severe Congenital Neutropenia, Myeloid Development and Leukemia

Severe Congenital Neutropenia

Severe congenital neutropenia (SCN) is characterized by arrested granulocyte development and disappearance of mature neutrophils ($<0.5 \times 10^9/L$) [30]. SCN is associated with mutations in *ELANE* [31,32], *WAS* [33], *HAX1* [34], *CSF3R* [35] and *GFI1* [36]. Targeted sequencing of these candidate genes in Severe Congenital Neutropenia International Registry patient samples recently revealed not only additional GFI1N382S and GFI1K403R mutations, but also novel non-synonymous SNPs—GFI1L400F and GFI1R412X [37]. Notably, mutations found in genes associated with SCN have not modeled the biology of the syndrome when introduced into the murine homolog, with the exception of the Gfi1N382S mutant [38].

Previously, the lineage choice between neutrophil and macrophage differentiation was suggested to be controlled by antagonism between the Cebpa and Pu.1 transcription factors [39]. Since both factors are required at some level for control of neutrophil and macrophage genes, neither Cebpa nor Pu.1 may resolve the fate of mixed lineage cells by suppressing expression of the opposing factor. Instead, Laslo et al. hypothesized that mixed granulocyte-monocyte lineage might be resolved by direct reciprocal antagonism of Gfi1 (putatively downstream of Cebpa) versus the Egr1 and Egr2 transcription factors and the Egr-corepressor Nab2 (downstream of Pu.1). In this study, Gfi1 directly repressed Egr2 [40] during late stages of myeloid development. In separate work, the expression of Gfi1N382S in the 32D cell line induced the expression of Cebpe resulting in G-CSF-stimulated cell death instead of differentiation [41]. Thus, Gfi1 has been thought to repress Egr2 [40] and Cebpe [41] to control granulopoiesis. However, Zarebski et al. showed that Gfi1N382S (which blocked granulopoiesis) and wild type Gfi1 (which stimulated granulopoiesis) similarly affected the expression of Egr1, Egr2, Nab2 and Cebpe in primary Lin⁻ cells [38]. Instead, Gfi1N382S selectively deregulated a subset of Gfi1 target genes including *Csf1* and *Csf1R*. GFI1-mutant SCN patient CD34⁺ bone marrow cells displayed over 60 fold deregulated expression of *CSF1* mRNA. This appears causative, because while expression of murine Gfi1N382S blocked granulopoiesis in murine Lin⁻ bone marrow cells, genetic or antibody ablation of *Csf1* expression restored granulopoiesis [38]. Next, Velu et al. defined microRNA (miR)-21 and miR196b as targets of Gfi1 repression and Gfi1N382S derepression [42]. Notably, Gfi1 regulates miR-21 and miR-196b in granulocytic-monocytic progenitors (GMP). Repression of these miRs is critical for allowing granulocytic differentiation as miR-21 functioned in a pro-monopoietic manner, while miR-196b exhibited anti-granulopoietic function *in vitro* (Fig. 1). Both miR were deregulated in a Gfi1N382S patient sample. Importantly, forced expression of miR-21 with miR-196b in *Gfi1*^{+/+} cells completely blocked G-CSF-stimulated granulopoiesis, phenocopying Gfi1N382S SCN patients [42]. The latter data illustrate microRNA as potent transducers of Gfi1 signaling; however, the relevant miR targets remain unknown.

Additional work linking Gfi1 to SCN-associated genes has demonstrated that Gfi1 regulates *ELANE* (encoding neutrophil elastase = NE) [36]. Recently, Salipante et al. discovered a novel protein, PFAAP5 (phosphonoformate immunoassociated protein-5), in a yeast-two hybrid screen linking both Gfi1 and NE. PFAAP5 was immunoprecipitated with both Gfi1 and NE and may bridge the molecules in the nucleus. In the presence of NE, PFAAP5 enhanced Gfi1's repression activity. Knockdown of PFAAP5 in human CD34⁺ cells lead to

decreased myeloid colony formation but not total CFU as is the case with Gfi1 knockdown [43]. Future work is necessary to determine how NE and PFAAP5 control Gfi1 target genes, and whether SCN-associated NE mutant proteins induce defects in Gfi1 repression mechanisms.

Myeloid Development & Leukemia

The HoxA9 transcription factor is of critical interest in human acute myeloid leukemia (AML). However, in-depth molecular analysis of HoxA9 gene target regulation and evaluation of their requirement for HoxA9-mediated oncogenesis is limited to *Pim1* [44], *c-Myb* [45] and *Flt3* [46,47]. Only *Pim1* [44] or *c-Myb* [45] impaired HoxA9 oncogenesis. Thus, the direct transcriptional network affected by endogenous HoxA9 (and therefore the HoxA9 mechanism of transformation) remains largely unknown.

Li-Kroeger et al. exploited *Drosophila* genetics to address a key mechanism of Hox factor function. Notably, the fly Hox factor complex is comprised of three proteins: (1) the Hox factor (Abd-A = Hox ortholog), (2) Extradenticle (Exd = Pbx ortholog) and (3) Homothorax (Hth = Meis ortholog). During analysis of the *rhomboid* (*rho*) gene cis-regulatory sequences, they made found: (1) Senseless (Sens = Gfi1 ortholog) binding sites can overlap Hox complex sites through the Sens “AATC” versus Exd “GATT” core sequence; (2) both Hox complexes and Sens can bind these sequences; and (3) transgenic-*lacZ* reporters, driven by one such sequence, faithfully recapitulated the expression of *rho* *in vivo*. Thus, the Hox complex competes with Sens for binding sites on target genes. If the Hox complex “wins”, the gene is activated. If the Sens protein “wins”, the gene is repressed [48].

In mammals, Horman et al. demonstrated that Gfi1 directly represses expression of *HoxA9*, *Meis1* and *Pbx1* during myelopoiesis [4]. Gfi1-mutant SCN patients and *Gfi1*^{-/-} mice display increased numbers of myeloid progenitors. Importantly, the *Gfi1*^{-/-} accumulation was dose-dependent upon alleles of *HoxA9*, genetically linking myeloid progenitor accumulation to this specific Gfi1 target gene. Epistasis experiments revealed a genetic relationship between *Gfi1* and *HoxA9*^{-/-} deficits in monopoiesis, which were corrected by *Gfi1* haploinsufficiency; however, no such relationship was discerned between *HoxA9* and *Gfi1*^{-/-} granulopoietic defects. These studies reveal that progenitors blocked in differentiation do not accumulate by default. Instead, the data demonstrate that transcriptional networks regulating granulopoietic programming and progenitor programming are separable, but commonly regulated by Gfi1 [4].

The accumulation of progenitors and high levels of HoxA9 with loss of Gfi1 may explain why SCN patients are at increased risk for the induction of AML. Indeed, HoxA9 expression was elevated in an SCN patient carrying the Gfi1N382S mutation. Although the limited lifespan of *Gfi1*^{-/-} mice precludes the induction of a slow-acting HoxA9-induced leukemia, the conditional Cre-mediated deletion of *Gfi1* and induction of K-RasG12D expression induced a transplantable AML in 17 days [4]. These data provide the first incisive proof that Gfi1 functions to suppress myeloid progenitor transformation.

Furthermore, an association between Gfi1 and human AML has recently been suggested. Khandanpour et al. sequenced the DNA of AML patients and identified a GFI136S versus GFI136N single nucleotide polymorphism (SNP) [49] previously seen in an SCN patient with persistent tetraploid mosaicism in the bone marrow [50]. Despite the minor change in the amino acid structure (OH vs COOH), the SNP showed increased risk for disease (OR=1.6), but no effect was seen on survival or other clinical manifestations or AML subgroup. The exact mechanism remains to be determined. Global gene expression changes were not found in AML patients with the mutant allele compared to AML patients with wild

type Gfi1 [49]. Future studies modeling this SNP in mice are expected to reveal specific Gfi1 target gene dysregulation.

In sum, Gfi1 is integral to multiple transcriptional programs regulating myeloid development: (1) the direct repression of target genes preventing neutrophil differentiation such as *Spfi1*, *Egr2*, *Csf1*, miR-21 and miR196b (the latter three are relevant to Gfi1N382S-induced SCN-associated defects), (2) the direct repression of *HoxA9*, *Meis1* and *Pbx1* in the transition to GMP. Putative target genes of Gfi1 and HoxA9 include *Cxcr4*, *Cdkn2B*, and *Spfi1* [4,51]. Thus, the transcriptional sequelae of Gfi1 loss of function is amplified by the derepression of the activator of a subset of Gfi1 target genes (HoxA9); creating a feed forward loop. Future work is necessary to utilize Gfi1-HoxA9 and Pu.1-Gfi1 antagonism as tools to dissect myelopoiesis and reveal critical targets controlling myelopoiesis. Notably, the two circuits interact (Table 1) (Fig. 1).

Gfi1 in Lymphoid Development and Function

T cell development

Gfi1 is required for T lymphopoiesis [52] as well as mature CD4⁺ and CD8⁺ T cell function and maintenance. Notably, while Gfi1 is required for naïve CD8⁺ (but not CD4⁺) T cell maintenance [53], Gfi1 appears to be essential for the CD4⁺ (but not CD8⁺) immune response [54]. Specifically, Gfi1 controls CD4⁺ T helper-2 (Th2) cell polarization, which requires expression of the IL-4-Stat6 target genes *Gfi1* and *Gata3* [55]. In human blood cells, GFI1 and GATA3 display significant concordant expression in CD4⁺ and CD8⁺ T cells as well as CD56⁺ natural killer (NK) cells [56]. Thus, the role of Gfi1 and Gata3 in cells outside the Th2 lineage needs to be explored more thoroughly. Within the Th2 lineage, Gfi1 and Gata3 biologically integrate the IL4 signal by enhancing proliferation and inhibiting apoptosis to expand presumptive Th2 cells [55]. Gfi1 physically associates with Gata3 preventing its ubiquitination and degradation [57]. One caveat to this explanation of Gfi1-Gata3 synergy is that the biological effects of Gfi1-Gata3 were dependent upon an intact Gfi1 SNAG repressor domain [55], and the role of SNAG associated proteins was not addressed.

Gfi1 controls Th2 lineage fidelity. Th2 cells express IL-4, -5 and -13, but not IFN γ . *Gfi1*^{-/-} cells show lower levels of repressive marks at the IFN γ locus, and decreased activating histone modifications at the IL-5 promoter, suggesting that in conjunction with Gata3, Gfi1 may also control Th2 effector function [57]. Moreover, loss of Gfi1 leads to increased production of the hallmark cytokine of Th17 cells, IL-17 α , as well as an increased plasticity in switching from Th2 to Th17 [58]. Furthermore, overexpression of Gfi1 prevented the formation of Th17 cells and IL-17 α production, but not ROR γ t expression. This result was independently replicated by another group [59]. Both groups demonstrated that Gfi1 levels were repressed upon TGF β signaling, a requisite of Th17 polarization [58,59]. Although *Gfi1*^{-/-} cells display dramatic differences in levels of histone 3 lysine 4 (H3K4) trimethylation at the *RORc* locus, no direct Gfi1 binding was identified. In contrast, the Gfi1 cofactor, Lsd1, occupied an intergenic region of the IL-17 gene in *Gfi1*^{+/+} (but not in *Gfi1*^{-/-}) Th2 primed cells. Thus, high levels of Gfi1 ensure the fidelity of Th2 polarization by repressing genes controlling alternative cell fates. Moreover, Th17 differentiation is initiated by a feed forward loop initiated by TGF β -signal-mediated repression of *Gfi1*, which releases IL-17 from Gfi1-mediated repression and induces IL-17-stimulated Th17 polarization [58].

CD103 is an important E-cadherin binding integrin found on human intraepithelial lymphocytes and on naturally occurring T-regulatory cells with high suppressive activity [60,61]. *Gfi1*^{-/-} cells lack CD103-locus-associated Lsd1, had higher levels of H3K4

trimethylation at the *CD103* locus, and had increased ability to prime iTregs, especially Foxp3⁺, CD103⁺ iTregs [58]. To delineate the functional relevance of these findings, Zhu et al. used an experimental model of multiple sclerosis (MS). MS is thought to be driven by Th17 biology. Loss of Gfi1 leads to increased Th17 polarization. However, the conditional *Gfi1* knockout mice showed delayed disease onset as well as decreased severity. This was likely due to the increased numbers of Foxp3⁺ CD103⁺ regulatory cells and their ability to modulate disease severity [58]. These findings may have some relevance to human disease in that 21 SNPs within the *GFI-EVI5-RPL5-FAM69A* locus were associated with MS in 732 patients. One SNP in the 17th intron of *EVI5* had a significant association with disease (P=0.008, OR=1.29; 95% CI=1.08-1.54) [62]. Notably, MMLV insertion mutagenesis in the last intron of *Evi5* activates murine *Gfi1* expression [2], but it is currently unclear whether the SNP functions similarly to affect human *GFI1* expression.

B-lymphopoiesis

Gfi1^{-/-} mice display profound deficits in B cell numbers due to a block in early lymphoid precursors [52]. IL-7 is critical for murine B lymphopoiesis. In CD8⁺ T cells, IL7 induces Gfi1 to antagonize GABPα activation of *IL7Ra* [63] and maintain naïve T cell pools [53]. In B cells, Gfi1 loss was associated with a deficit in response to IL7 [64], providing the possibility that Gfi1 is a downstream effector of IL7 signaling. More recently, a transcriptional network was suggested to explain *Gfi1*^{-/-} defective B lymphopoiesis [16]. Specifically, the “C/Ebpα-Gfi1 versus Egr2-Pu.1” circuit [40] was revised to posit that in multipotent progenitors (MPPs) the transcription factor Ikaros upregulates Gfi1 expression. In this “Ikaros-Gfi1 versus Egr2-Pu.1” circuit, Ikaros replaces Cebpα, but Gfi1 still represses Egr2 to prevent myeloid differentiation and allow lymphoid priming. Previously, Gfi1 was shown to physically bind Pu.1 protein and antagonize Pu.1 target gene activation [65]. Using Pu.1 ChIP-on-chip analysis, Spooner et al showed that 19% of Pu.1-bound regions contained putative binding sites for both Gfi1 and Pu.1 within 10bp of each other, including the promoter of the Pu.1-encoding gene (*Sfp1*). Global Pu.1/Gfi1 binding was not proven, but the suggestion is that direct antagonism between Pu.1 and Gfi1 is possible. In agreement with this, haploinsufficiency of *Sfp1* on a *Gfi1*^{-/-} null background, partially restored B cell potential [16]; however, this analysis does not delineate between Gfi1 repression of *Sfp1* versus direct Gfi1 antagonism of Pu.1 target genes.

Gfi1 is not expressed in mature unchallenged B cells. Interestingly, *Gfi1*, *Egr1*, *Egr2* and *Nab2* are coordinately upregulated subsequent to B cell receptor (BCR) ligation through an ERK-dependent tolerogenic signal [66]. *Gfi1*^{-/-} B-cell-driven autoimmunity has been reported [67], but not confirmed [68]. Instead, the second group noted *Gfi1*^{-/-} B cells display increased levels of serum IgG antibodies after antigen challenge. Gfi1 did not bind the promoters of any of the *Iγ* loci [68]. Instead, the authors posit a 32-fold increase in *TGFβ1* transcript levels as causative; however, this only correlated to ~2-fold increase in TGFβ1 protein following lipopolysaccharide (LPS)-challenge [68]. Thus, tolerogenic BCR signaling induces Gfi1 which may control IgG expression; however, the functional ramifications of Gfi1 in mature B cells needs to be resolved.

Gfi1 in Lymphoid Leukemia

Dabrowska et al. demonstrated how MMLV insertion mutagenesis overcomes miR targeting to activate Gfi1 expression [69]. While insertion sites in the Gfi1 3' UTR were known to lead to increased transcript levels [2,70], these insertions also truncate the *Gfi1* transcript to eliminate binding sites for a number of miRs, including miR-155 and miR-10a [69]. Luciferase sensor assays confirmed targeting of Gfi1 3'UTR by the miR [69]. These new findings implicate miRs in controlling Gfi1 protein levels in both normal and oncogenic settings, and suggest future work to delineate proteosomal degradation [71,72] versus miR

mechanisms to control Gfi1 levels. Interestingly, they also showed leukemia-specific *Gfi1* mRNA alternative splicing, but did not document transcript abundance [69].

Using a yeast 2-hybrid screen, Gfi1 and Miz-1 (Myc-interacting zinc finger protein-1) were found to interact and form a complex with c-Myc [73]. This interaction was present in multiple cell lines and could repress target genes *Cdkn2B* (p15) and *Cdkn1B* (p27) in luciferase assays. Increased expression of *Cdkn2B* (a putative HoxA9 target gene) was observed in *Gfi1*^{-/-} bone marrow [73]. Upregulation of *Cdkn2B* may be augmented by deregulated expression of HoxA9, Meis1 and Pbx1 in *Gfi1*^{-/-} cells [4]. The repression of *Cdkn2B* might explain the collaboration between Gfi1 and Myc in lymphoid malignancies, but this remains to be proven.

Hematopoietic-relevant lessons learned from Gfi1 in non-hematopoietic tissues

While Gfi1 repression of target genes is well known, work from the *Drosophila* field suggests that Gfi1 may synergize with other transcription factors to activate target genes, and this may be relevant to humans with specific granule deficiency (SGD). Studies from three groups show that *Drosophila* Senseless can function as a potent transcriptional co-activator. In developing sensory precursor cells, the basic Helix-Loop-Helix (bHLH) factors Achaete, Scute, and Atonal stimulate gene expression by recruiting Sens through its zinc finger motifs [74,75]. Additionally, Sens stimulates the ability of the Orthodenticle homeodomain transcription factor to activate *rhodopsin* gene expression within photoreceptor cells [76]. In humans, Khanna-Gupta et al. demonstrated that a patient with SGD who was wild type for C/EBP ϵ , had abnormally low levels of GFI1. She further showed that GFI1 and C/EBP proteins bind in close proximity in the promoter of the neutrophil collagenase promoter, and that cotransfection of GFI1 and C/EBP ϵ activated a neutrophil-collagenase-promoter driven reporter [17]. While the mechanism of putative Gfi1 coactivator function is unknown, the effect appears to span evolution.

In a pathway similar to its *Drosophila* ortholog Senseless, Gfi1 is essential for the bHLH-initiated differentiation and survival of several solid organ cell types. A common mechanism is antagonism to Notch. The atonal homologs Atoh1 and Atoh7 function upstream of Gfi1 in the inner ear, intestine, and retina, respectively [8,10,77]. In the nervous system Gfi1 is required for differentiation and survival of the mechanosensory hair cells of the inner ear, and for survival of cochlear ganglion neurons and cerebellar purkinje cells [10,78]. In the small intestine and colon, Gfi1 is expressed in progenitor cells and directs appropriate differentiation of enteroendocrine, goblet and Paneth cells [8]. In the lung, Gfi1 functions downstream of the achaete/scute homolog Ascl1, and is required for pulmonary neuroendocrine cell differentiation [9]. In each of these contexts, Gfi1 functions in concert with its respective bHLH factor to oppose the activity of Notch, and thereby direct differentiation of a specific cell fate [78,79]. Future work is necessary to discern a functional relationship between Gfi1 and Notch signaling in hematopoietic cells.

Conclusion

Complex transcriptional circuits promoting differentiation and proliferation are intimately linked to Gfi1. Multiple disease states are observed with Gfi1 gain and loss of function such as severe congenital neutropenia, non-immune chronic idiopathic neutropenia of adults, specific granule deficiency and perhaps AML and T-ALL. However, large gaps in knowledge still surround Gfi1 critical targets and cofactors. Defining these targets and partners will potentiate methods to manipulate these proteins for clinical intervention.

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Figure 1.

Integrated view of Gfi1 transcriptional networks. Using a linear model of hematopoiesis, the published role of Gfi1 in each cell type is outlined by showing upstream activators and downstream targets of Gfi1. Solid lines: Direct targets, as confirmed by ChIP, EMSA or other physical binding data. Dotted lines: indirect targets, suggested by gene expression data. Green lines: relevant to SCN-related Gfi1N382S mutation. Blue lines: Gfi1 active circuit. Red lines: antagonism to Gfi1. The requirement for Gfi1 in DN T cells is shown with blue fill.

Table 1

Potential mechanisms of Gfi1–HoxA9/Pu.1 antagonism

Mechanism	Effect
1. Gfi1 binding to <i>HoxA9</i> , <i>Meis1</i> , <i>Pbx1</i> promoters.	Decreased HoxA9, Meis1 and Pbx1 protein expression and indirect repression of Hox-complex targets (putatively <i>Spfi1</i>)
2. Gfi1 competing for HoxA9-Pbx1-Meis1 binding sites (not proven in mammals).	Direct repression of a subset of HoxA9 target genes with composite Gfi1-HoxA9 binding sites.
3. Gfi1 binding to <i>Spfi1</i> promoter	Decreased Pu.1 protein
4. Gfi1 binding to <i>Csf1R</i> promoter (a Pu.1 target)	Decreased Csf1R protein
5. Gfi1-Pu.1 protein-protein interaction	Repression of Pu.1 target genes (e.g. <i>Csf1R</i>)
6. Gfi1-Pu.1 bind in close proximity on a subset of common target genes (not proven)	Repression of a subset of Pu.1 target genes
7. Pu.1 binding promoters of <i>Egr-1</i> , <i>Egr-2</i> , <i>Nab2</i>	Increased expression of <i>Egr-1</i> , <i>Egr-2</i> , <i>Nab2</i> and subsequent repression of <i>Gfi1</i>